Marine Biology 118, 377–382 (1994)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration

C. Clemmesen*

Institut für Hydrobiologie und Fischereiwissenschaft, Universität Hamburg, Germany

Received: 14 September 1993 / Accepted: 28 October 1993

Abstract. RNA/DNA ratios in individual herring (*Clupea harengus*) larvae (collected from Kiel Bay, Baltic Sea, in 1989) were measured and proved suitable for determining nutritional status. Significant differences between fed and starving larvae appeared after 3 to 4 d of food deprivation in larvae older than 10 d after hatching. The RNA/DNA ratio showed an increase with age or length of the larvae and was less pronounced in starving larvae compared to fed larvae. The individual variability of RNA/DNA ratios in relation to larval length of fed larvae and of larvae deprived of food for intervals of 6 to 9 d is presented. Based on the length dependency and the individual variability found within the RNA/DNA ratios, a laboratory calibration is given to determine whether a larva caught in the field has been starving or not. An example for a field application is shown.

Introduction

High mortality rates during early life history stages most possibly determine the size and fluctuations of a year class. It is assumed that either the lack of food or a mismatch in larval fish and food organisms distribution is a principal cause of poor year class strength (Hjort 1914, Hunter 1976, Lasker 1987). It has been shown that the ratio of ribonucleic acid (RNA) to deoxyribonucleic acid (DNA) is a useful indicator of nutritional condition. Nucleic acids play a major role in growth and development. The amount of DNA, the carrier of the genetic information, is quasi-constant in somatic tissues, and tissue concentrations therefore reflect cell numbers. The amount of RNA in the cell is directly proportional to the amount of protein synthesis occurring. The relationship between RNA and DNA is an index of the cell's metabolic intensity and has been used to measure recent growth in fishes (see review by Bulow 1987). It has proven a useful indicator of nutritional condition as has been shown in several larval fish studies (Buckley 1980, 1981, 1984, Martin et al. 1985, Fukuda et al. 1986, Buckley and Lough 1987, Clemmesen 1987, Raae et al. 1988, Robinson and Ware 1988, Westerman and Holt 1988). Previous studies measuring RNA/DNA ratios in larval fish used a method which required a minimum of 800 µg dry weight per sample (Buckley 1979), so pooled samples had to be analyzed, and estimates of the individual variability in RNA/DNA ratios were impossible. But such estimates could be essential for detecting starving individuals in the field in order to determine whether starvation plays a major role in the recruitment process. By use of a now available highly sensitive fluorimetric method which allows the determination of the RNA/DNA ratios of individual larvae (Clemmesen 1988, 1993) the effect of age and size on the RNA/DNA ratios of fed and starving larvae can be analysed. Laboratory-reared herring (*Clupea harengus*) larvae were fed or deprived of food for intervals of 3 to 9 d in relation to age and size in order to produce a calibration for the determination of the nutritional status of fish larvae from the field. A length-dependent relationship with critical RNA/DNA levels for starved and fed laboratory-reared herring larvae is given.

Materials and methods

Larval material

Adult herring were captured alive in 1989 by using a fish trap in Kiel Bay (Baltic Sea). The fish were stripped, and the fertilized eggs were incubated at 9.2 °C. Larvae were reared in 100 and 400 l square tanks. Temperature varied between 12.0 and 16.7 °C with a mean temperature of 14.4 °C. One group of larvae was offered rotifers (*Brachionus plicatilis*) directly after hatching until the end of the experiment (Day 65). Freshly hatched *Artemia* spp. nauplii (San Francisco brand) were added to the diet from Day 19 to 65. Mean concentrations of 5.0 *B. plicatilis* ml^{-1} and 1.0 *Artemia* spp. ml^{-1} were adjusted daily. The other group was deprived of food for intervals of 3 to 9 d before sampling. Larval samples were taken prior to feeding. To evaluate the quality of the rearing facilities,

* *Present address*: Institut für Meereskunde Kiel, Düsternbrooker Weg 20, D-24105 Kiel, Germany

RNA/DNA ratios of herring eggs and larvae fed with *B. plicatilis* and *Artemia* spp. at a density of 3.0 and 0.5 organisms ml^{-1} were analysed to observe whether larvae have RNA/DNA ratios present in the eggs. It was assumed that the ratios of the eggs would reflect a good condition. One to two eggs were needed to determine the RNA/DNA ratio. As an indirect and easy-to-measure indicator of age the length of the larvae was determined prior to analysis.

Measurements of RNA/DNA ratios

Determination of RNA and DNA contents was performed according to the fluorescence technique described by Clemmesen (1988) with some modifications (Clemmesen 1993). The analytical procedure involves purification of larval tissue homogenates and subsequent fluorescence-photometric measurement using a specific nucleic acid fluorescent dye, ethidium bromid (EB), for DNA and RNA. In order to measure the DNA content of a sample, RNA was enzymatically digested by RNase and the remaining DNA was determined with EB.

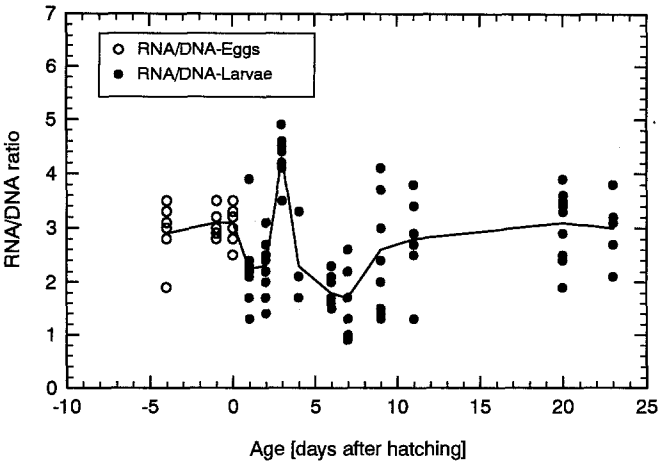

Fig. 1. *Clupea harengus.* Individual RNA/DNA values of herring eggs and fed larvae. Line connects the mean values of each age group. 0: eggs at the day of hatching

Results

The RNA/DNA ratios of herring eggs and larvae are presented in Fig. 1. Larvae were fed from the day of hatching until the end of the experiment. The RNA/DNA ratios of the eggs increased slightly towards hatching and had a mean value of 3.0. The RNA/DNA ratios of the larvae showed a decrease in the ratio right after hatching, followed by an increase at Day 3 and a renewed decrease at Day 5. This might be due to the high mobilisation of the yolk reserves just after hatching followed by a reduced metabolic activity at the end of the yolksac stage. Larvae had RNA/DNA ratios in the range of 1.3 to 4.9. The ratio stabilized at about Day 11 with a mean of 3.0, which reflected the values found in the eggs.

A comparison of RNA/DNA ratios of starved and fed herring larvae is shown in Fig. 2a, b. In the first days after hatching fed larvae also showed RNA/DNA ratios in the range found for starving larvae (Fig. 2a), therefore no separation was possible. For larvae 10 d and older, deprivation of food lead to a significant decrease in the RNA/DNA ratios (Fig. 2b) in comparison to fed larvae after 3 to 4 d (ANOVA, $p<0.05$). An increase in the starvation interval resulted in a further decrease in the RNA/DNA ratio. It was reduced to half after 6 to 7 d of food deprivation, and showed less variability within the RNA/DNA ratios compared to the ratios of larvae starved for 3 to 4 d. The RNA/DNA ratios of fed larvae showed a tendency to increase with the age of the larvae.

The RNA and DNA contents (Fig. 3a, b) and the RNA/DNA ratios (Fig. 4) of food-deprived larvae were compared with fed larvae in relation to larval length. The RNA and DNA contents as well as the RNA/DNA ratios of starving larvae were combined in two groups: larvae starved for 3 to 4 d and larvae starved for 6 to 9 d. A comparison of the DNA content per length ($\mu g\ mm^{-1}$) in relation to larval length didn't show a significant difference whether larvae were fed or starving (Fig. 3a). In contrast, the RNA content per length ($\mu g\ mm^{-1}$) showed significantly lower RNA values for starving larvae (ANOVA, $p<0.05$), meaning that the RNA content per length at a given length class is reduced in starving

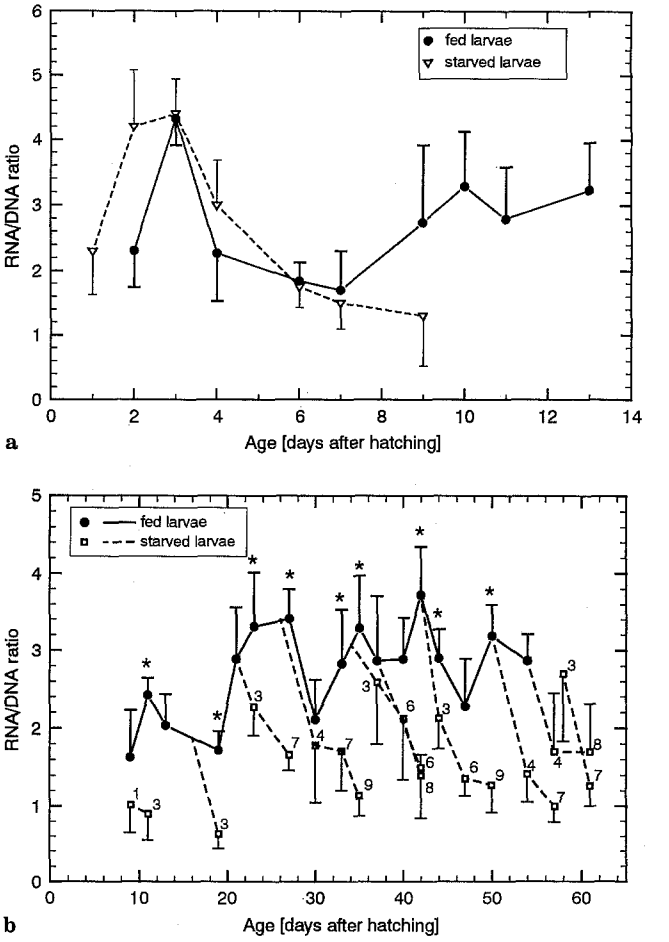

Fig. 2. *Clupea harengus.* RNA/DNA ratios of fed and starving herring larvae in relation to larval age; (a) age 1 to 13 d (b) age 9 to 61 d. Data points are means of 5 to 15 individually determined RNA/DNA ratios. Vertical bars show standard deviation, for better clarity, only given one-sided. (b) Numbers denote length of starvation time in days prior to analysis. Asterisks refer to significant differences between means of starved and fed larvae of the same age (Mann-Whitney *U*-test, $p<0.05$)

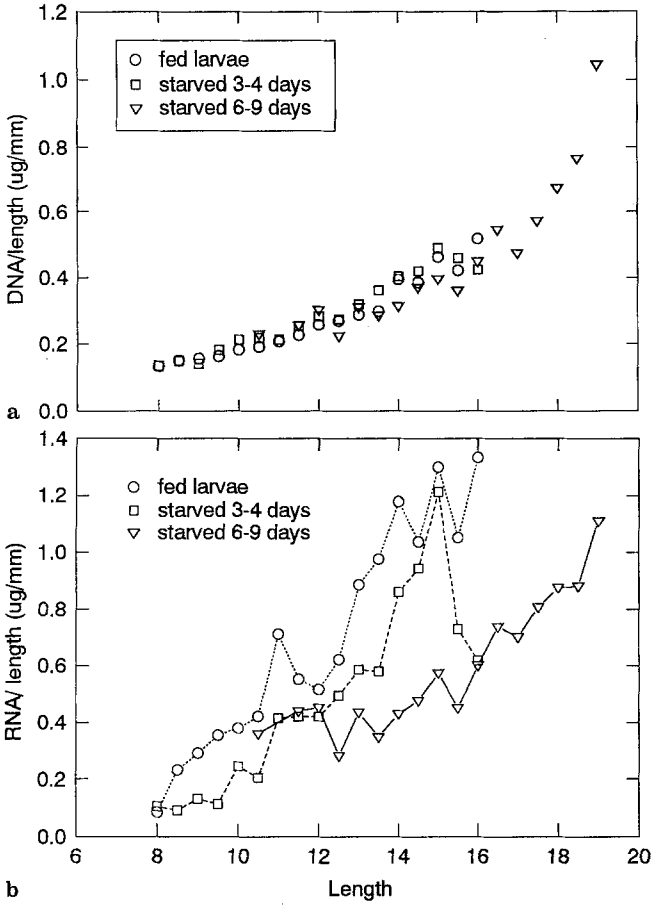

Fig. 3. *Clupea harengus.* Relation between (a) mean DNA-content per length ($\mu g\,mm^{-1}$) and (b) mean RNA-content per length ($\mu g\,mm^{-1}$) and larval length. Results from fed larvae and larvae deprived of food for 3 to 4 or 6 to 9 d are given. Lines connect the mean values of each nutritional group

larvae and decreases further with an increase in the length of starvation time (Fig. 3b). The consequence is a significantly reduced RNA/DNA ratio (ANOVA, $p<0.05$) in starving larvae compared to fed larvae (Fig. 4). All three nutritional groups showed an increase in the RNA/DNA ratio with the length of the larvae, most obvious in the fed group and less pronounced in the starving groups. The variability within the RNA/DNA ratios of a given length class decreased with increasing starvation time. Larvae starved for 6 to 9 d showed a decreased length dependency and less variability compared to larvae starved for 3 to 4 d (Fig. 4). When comparing the relation between ratio and length and ratio and age, the variability within the RNA/DNA ratios of the length groups was reduced since the consideration of larval length compensates for the differences in growth occurring within the age groups.

Fig. 5 shows the individual variability of the RNA/DNA ratios and the length dependency of the ratios for herring larvae fed constantly during the course of the experiment and for those starved for 6 to 9 d. For both groups linear regression models and the 95% confidence intervals of the regression were fitted to the data. The RNA/DNA ratios of fed larvae were spread over a wide range and some overlap with the values found in starved larvae was shown.

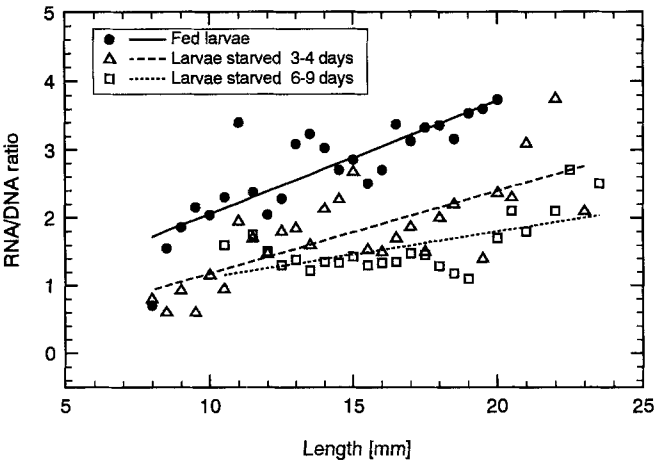

Fig. 4. *Clupea harengus.* Relation between RNA/DNA ratios (mean values) and larval length. Results from fed larvae and larvae deprived of food for 3 to 4 or 6 to 9 d given. Lines fitted by linear regression analysis

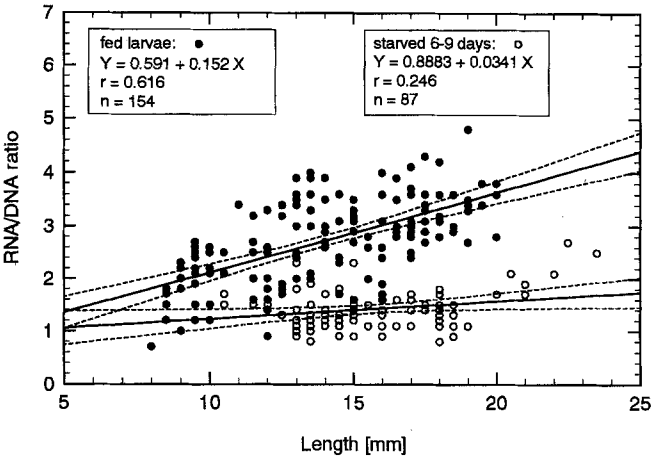

Fig. 5. *Clupea harengus.* Relation between RNA/DNA ratios (individual values) and larval length for larvae fed constantly during the course of the experiment and for larvae deprived of food for 6 to 9 d. Continuous lines fitted by linear regression analysis. 95% confidence intervals of the regressions given by dashed lines

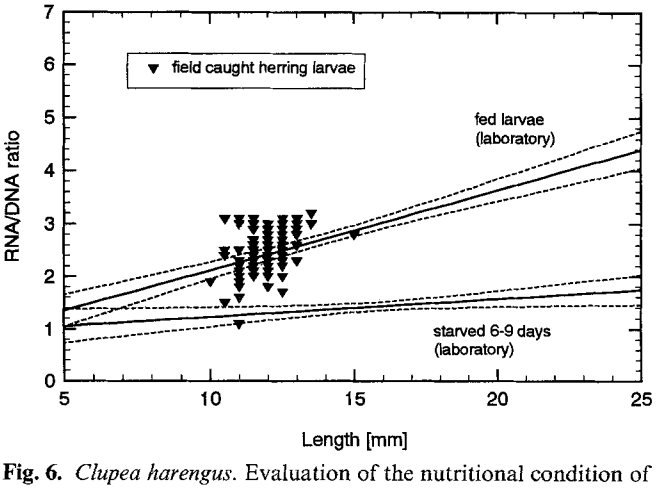

Fig. 6. *Clupea harengus.* Evaluation of the nutritional condition of herring larvae caught in the English Channel in January 1986. Individual RNA/DNA values ($n=148$) fitted to data determined on fed and 6- to 9-d starved laboratory reared herring larvae

To evaluate whether larvae from the field were starving or not their RNA/DNA values were compared with RNA/DNA ratios of laboratory-reared herring larvae deprived of food for 6 to 9 d. The condition of the 10 to 15 mm (SL) herring larvae caught during an Int. Council Explor. Sea (I.C.E.S.) survey in the English Channel in January 1986 was evaluated by plotting their RNA/DNA ratios into the regression model of starved and fed laboratory-reared herring larvae (Fig. 6). 1.4% of the larvae from the English Channel had ratios below or within the 95% confidence interval of the regression of 6- to 9-d starved larvae and were therefore determined to be starving. Based on the regression line of RNA/DNA ratios of fed laboratory larvae, 41.2% of the field larvae had RNA/DNA ratios below these levels.

Discussion

The aim of the laboratory experiments was to show the suitability of the RNA/DNA ratio to determine the nutritional status of individual fish larvae and to establish an application to evaluate the nutritional condition of larvae caught in the field. Therefore, a laboratory calibration of the RNA/DNA ratio was performed to determine whether a larva was well fed or suffering from starvation prior to sampling. The laboratory-rearing facilities used provided larvae in a condition equivalent to the condition found in the eggs. It, therefore, can be assumed that the facilities used should allow good larval development. The mean value of 3.0 found in herring eggs is in good agreement with a RNA/DNA ratio of 3.2 given by Buckley (1981) for winter flounder eggs.

With the analytical nucleic acid method used here individual determination of condition and variability within what is theoretically an equally treated group could be investigated. Significant differences in the RNA/DNA ratios of fed and starving larvae were found after 3 to 4 d of food deprivation. An extension of the period of starvation resulted in a further decrease in the ratio. The influence of shorter starvation times could not be measured with the methodology applied here. Generally, the ribosomes (RNA) are able to react to changes in nutritional condition within hours by decreasing their activity. But a reduction in the ribosome number and therefore RNA content at the temperature range used in the present study only occurs after longer periods of starvation in the range of days (Henshaw et al. 1971, Millward et al. 1976, Lied et al. 1982, Houlihan 1988). 3 to 4 d of starvation led to a nutritional limitation from which the larvae were able to recover after 4 to 7 d when food was available again (Clemmesen-Bockelmann 1992). Studies by Blaxter and Ehrlich (1974) showed that herring larvae reached the 'point of no return', depending on age and temperature, after 6 to 15 d of food deprivation. Therefore, 6 to 9 d starvation as used in the present study represents a time period important in recruitment studies.

There was no significant difference in RNA/DNA ratios between fed and starving herring larvae from hatching up to an age of 10 d. Although sufficient food was available, fed larvae shortly after hatching showed reduced RNA/DNA ratios in the same range as those found for starved larvae. This seems to be due to problems resulting from the change of internal to external nutrition. During this transitional phase developmental and physiological changes such as the opening of the mouth and esophagus, development of liver, gallbladder and pancreas (Govoni 1980, O'Connell 1981, Govoni et al. 1986), the production of proteolytic digestive enzymes and the resorption ability of the gut (Dabrowski 1984, Ueberschär 1985, Deplano et al. 1991) must take place. Delayed development or innate genetic defects could explain some of the low RNA/DNA ratios within the fed group. The problem of not being able to distinguish fed and starving larvae in the first few days after hatching was also pointed out by other authors using different indicators of nutritional condition (O'Connell 1976, Martin et al. 1984, Pedersen et al. 1987, 1990). Low RNA/DNA ratios in both groups, therefore, are not due to RNA/DNA-specific methodological problems but rather to a natural phenomenon caused by a change in nutritional uptake.

By using a nucleic acid analysis sensitive enough for individual larvae, the length dependency of the RNA/DNA ratio could be analysed for the first time. An increase in the RNA/DNA ratio with length or age of the larvae in a group being treated equally with respect to nutrition was unexpected. A stable RNA/DNA ratio characteristic for well nourished larvae independent of length may only be reached when a metabolic balance is achieved. Studies by Fukuda et al. (1986) showed that herring larvae had to reach 30 mm to show a constant level of glycogen, phospholipids and proteins in relation to dry weight, indicating that metabolic or physiological changes were occurring in smaller larvae. An increase in the ratio with age or length of the larvae may be effected by cells growing without the DNA content increasing (hypertrophy), which is specific to locomotory muscle DNA (Goss 1966). In future studies this could be further evaluated by determining the C/DNA ratios (Anger and Hirche 1990) or by measuring μg dry weight μg^{-1} DNA as an index of cell size (Ota and Landry 1984). The increase in RNA/DNA ratios with an increase in the length of the larvae in the group deprived of food for 6 to 9 d is probably due to the fact that the starvation potential is longer in older (larger) larvae. Larger larvae need longer time periods to reach lower RNA/DNA ratios. It is postulated that a further increase in starvation time in older larvae will result in a minimum RNA/DNA level which will be independent of the larva's length. There are indications in the literature that RNA/DNA ratios reached after starvation periods in the time range of days to weeks result in a value of ca. 1 near the 'point of no return' (Table 1).

Based on the laboratory experiments presented, two general possibilities for the determination of the nutritional condition exist. The RNA/DNA ratios of herring larvae of unknown condition can be compared with ratios found in fed larvae or with RNA/DNA ratios determined in starving larvae. Since it is easier to define starvation situations under laboratory conditions in which food is withdrawn completely rather than to define good

Table 1. RNA/DNA ratios of different species of starved fish larvae

Species	Starvation time (d)	Mean RNA/DNA	Source
Ammodytes americanus	7	1.5	Buckley et al. 1984
	14	1.0	
Clupea harengus	9	1.2	Ueberschär and
	13	1.1	Clemmesen 1992
Clupea harengus	9	1.3	Clemmesen 1987
Morone saxatilis	12	1.2	Wright and Martin 1985
Pleuronectes platessa	14	1.0	Hovenkamp 1990
Scophthalmus maximus	7	1.3	Clemmesen 1987
Solea solea	9	1.1	Richard et al. 1991
Theragra chalcogramma	7	1.0	Canino et al. 1991

feeding conditions, it seems advisable to compare RNA/DNA values from larvae caught in the field with ratios found in larvae deprived of food in the laboratory. It is also more important for the recruitment problem to show food limitation or badly nourished larvae than to define well fed larvae. Further, the RNA/DNA ratios in starving larvae in the present study showed a less pronounced length dependency than in fed larvae and a reduced individual variability. It also should be noted that the RNA/DNA ratios found in 6- to 9-d starving larvae in the present study are in good agreement with other values determined in different species of starving fish larvae (Table 1). There seems to be a species-independent minimum RNA/DNA value around 1 necessary for survival. The author is therefore confident that the laboratory-determined RNA/DNA ratios of starving herring larvae can be used to determine starvation of field-caught larvae and could also be used to evaluate the condition of larvae other than herring. In comparison the RNA/DNA values of well fed fish larvae from laboratory situations are less reliable.

All 'critical values' for the evaluation of condition published so far have used a length- and age-independent RNA/DNA ratio which did not account for the natural variability within a starving group, since the methods applied did not allow for individual examination (Buckley 1984, Martin et al. 1984, Robinson and Ware 1988). The first restrictions on the use of these critical values were made by Clemmesen (1989), who described two critical levels in dependence of larval age. In the present study critical RNA/DNA values which take length dependency and individual variability into account are shown. This resulted, for example, in mean RNA/DNA values of 1.2 for 10-mm larvae starved for 6 to 9 d compared to a mean RNA/DNA ratio of 1.7 for 23-mm larvae.

Based on constant critical values which have been used in previous studies, an evaluation of the nutritional situation of larvae from field samples would be biased compared to the length-dependent critical values presented here. The number of starving larvae in the small length groups would be over-estimated, the number of larger starving larvae would be under-estimated based on a constant level. This discrepancy is even more important if the condition of the larvae is to be evaluated based on the regression model found for fed larvae. Therefore, it is necessary to measure the larva's length, i.e., age, or developmental stage and to take this into consideration when the nutritional condition of the larva is to be evaluated based on RNA/DNA ratios.

The effect of temperature was not determined in the present study, but has to be further evaluated, since it influences physiological processes and has been shown to effect RNA/DNA ratios. Higher RNA tissue concentrations at lower temperatures may be a compensatory mechanism for lower RNA activity (Buckley 1982, Goolish et al. 1984, Mathers et al. 1992).

The use of a highly sensitive fluorescence method to determine individual RNA/DNA ratios and the validity of the RNA/DNA ratio to describe the nutritional situation of a fish larvae have proven useful. It is postulated that the presented critical RNA/DNA values make it possible to evaluate whether a larvae caught in the field is starving or not.

Acknowledgement. I thank the staff of the fishery department of the University of Marine Sciences in Kiel for their support. Special thanks to B. Ueberschär for excellent team work when rearing the larvae and for helpful discussion throughout the study. I am indebted to Prof. W. Nellen and Prof. D. Schnack for their support. This manuscript is part of a thesis submitted to the University of Hamburg in partial fulfillment of the requirements for the Ph.D. degree. Financial support was provided by the Bundesminister für Forschung und Technologie grant no. MFE 0541/9.

References

Anger, K., Hirche, H.-J. (1990). Nucleic acids and growth of larval and early juvenile spider crab, *Hyas araneus*. Mar. Biol. 105(3): 403–411

Blaxter, J. H. S., Ehrlich, K. F. (1974). Changes in behaviour during starvation of herring and plaice larvae. In: Blaxter, J. H. S. (ed.) Early life history of fish. Springer Verlag, Heidelberg, p. 575–588

Buckley, L. J. (1979). Relationship between RNA/DNA ratio, prey density and growth rate in Atlantic Cod (*Gadus morhua*) larvae. J. Fish. Res. Bd Can. 36: 1497–1502

Buckley, L. J. (1980). Changes in the ribonucleic acid, deoxy ribonucleic acid, and protein content during ontogenesis in winter flounder, *Pseudopleuronectes americanus*, and the effect of starvation. Fish Bull. U.S. 77: 703–708

Buckley, L. J. (1981). Biochemical changes during ontogenesis of cod (*Gadus morhua* L.) and winter flounder (*Pseudopleuronectes americanus*) larvae. Rapp. P.-v. Réun. Cons. int. Explor. Mer 178: 547–552

Buckley, L. J. (1982). Effects of temperature on growth and biochemical composition of larval winter flounder (*Pseudopleuronectes americanus*). Mar. Ecol. Prog. Ser. 8: 181–186

Buckley, L. J. (1984). RNA/DNA ratio: an index of larval fish growth in the sea. Mar. Biol. 80: 291–298

Buckley, L. J., Lough, R. G. (1987). Recent growth, chemical composition and prey field of haddock (*Melanogrammus aeglefinus*) and cod (*Gadus morhua*) larvae and post larvae on Georges Bank, May 1983. Can. J. Fish. aquat. Sciences 44: 14–25

Buckley, L. J., Turner, S. I., Halavik, T. A., Smigielski, A. S., Drew, S. M., Laurence, G. C. (1984). Effects of temperature and food availability on growth, survival and RNA/DNA ratio of larval sand lance (*Ammodytes americanus*). Mar. Ecol. Prog. Ser. 15: 91–97

Bulow, F. J. (1987). RNA-DNA ratios as indicators of growth in fish: a review. In: Summerfeld, R. C., Hall, G. E. (eds.) Age and growth in fish. Iowa State University Press, Ames, Iowa, p. 45–64

Canino, M. F., Bailey, K. M., Incze, L. S. (1991). Temporal and geographic differences in feeding and nutritional condition of walleye pollock larvae *Theragra chalcogramma* in Shelikof Strait, Gulf of Alaska. Mar. Ecol. Prog. Ser. 79: 27–35

Clemmesen, C. (1987). Laboratory studies on RNA/DNA ratios of starved and fed herring (*Clupea harengus*) and turbot (*Scophthalmus maximus*) larvae. J. Cons. int. Explor. Mer 43: 122–128

Clemmesen, C. (1988). A RNA and DNA fluorescence technique to evaluate the nutritional condition of individual marine fish larvae. Meeresforsch. 32: 134–143

Clemmesen, C. (1989). RNA/DNA ratios of laboratory-reared and wild herring larvae determined with a highly sensitive fluorescence method. J. Fish. Biol. 35 (Suppl. A): 331–333

Clemmesen, C. (1993). Improvements in the fluorimetric determination of the RNA and DNA content of individual marine fish larvae. Mar. Ecol. Prog. Ser. 100: 177–183

Clemmesen-Bockelmann, C. (1992). Das RNA/DNA Verhältnis als Indikator für den Ernährungszustand von Fischlarven. Dissertation der Universität Hamburg, Hamburg

Dabrowski, K. (1984). The feeding of fish larvae: present "state of the art" and perspectives. Reprod. Nutr. Dév. 24: 807–833

Deplano, M., Connes, R., Diaz, J. P., Barnabe, E. G. (1991). Variation in the absorption of macromolecular proteins in larvae of the sea bass *Dicentrarchus labrax* during transition to the exotrophic phase. Mar. Biol. 110: 29–36

Fukuda, M., Nakano, H., Yamamoto, K. (1986). Biochemical changes in Pacific herring during early developmental stages. Hokkaido Daigaku Fac. Fish Bull. 37: 30–37

Goolish, E. M., Barron, M. G., Adelman, I. R. (1984). Thermoacclinatory response of nucleid acid and protein content of carp muscle tissue: influence of growth rate and relationship to glycine uptake by scales. Can. J. Zool. 62: 2164–2170

Goss, R. J. (1966). Hypertrophy versus hyperplasia. Science, N.Y. 153: 1615–1620

Govoni, J. J. (1980). Morphological, histological and functional aspects of alimentary canal and associated organ development in larval *Leiostomus xanthurus*. Rev. Can. Biol. 39: 69–80

Govoni, J. J., Boehlert, G. W., Watanabe, Y. (1986). The physiology of digestion in fish larvae. Envir. Biol. Fish 16: 59–77

Henshaw, E. C., Hirsch, C. A., Morton, B. E., Hiatt, B. M. (1971). Control of protein synthesisin mammalian tissues through changes in ribosome activity. J. Biol. Chem. 246: 436–446

Hjort, J. (1914). Fluctuations in the great fisheries of Northern Europe viewed in the light of biological research. Rapp. P.-v. Réun. Cons. Perm. int. Explor. Mer. 160: 1–228

Houlihan, D. F., Hall, S. J., Gray, C., Noble, B. S. (1988). Growth rates and protein turnover in the Atlantic cod, *Gadus morhua*. Can. J. Fish. aquat. Sciences 45: 951–964

Hovenkamp, F. (1990). Growth differences in larval plaice *Pleuronectes platessa* in the Southern Bight of the North Sea as indicated by otolith increments and RNA/DNA ratios. Mar. Ecol. Prog. Ser. 58: 205–215

Hunter, J. R. (1976). Report of a colloquium on larval fish mortality studies and their relation to fisheries research, Jan. 1975. NOAA tech. Rep. U.S. Dep. Commerce NMFS Circ. 395: 1–5

Lasker, R. (1987). Use of fish eggs and larvae in probing some major problems in fisheries and aquaculture. Am. Fish. Soc. Symp. 2: 1–16

Lied, E., Lund, B., Von der Decken, A. (1982). Protein synthesis in vitro by epaxial muscle polyribosomes from cod, *Gadus morhua*. Comp. Biochem. Physiol. 72B: 187–193

Martin, F. D., Wright, D. A., Means, J. C. (1984). Fatty acids and starvation in larval striped bass (*Marone saxatilis*). Comp. Biochem. 77: 785–790

Martin, F. D., Wright, D. A., Means, J. C., Setzler-Hamilton, E. F. (1985) Importance of food supply to nutritional state of larval striped bass in the Potomac river estuary. Trans. Am. Fish. Soc. 114: 137–145

Mathers, E. M., Houlihan, D. F., Cunningham, M. J. (1992). Nucleic acid concentrations and enzyme activities as correlates of growth rate of the saithe *Pollachius virens*: growth rate estimates of open-sea fish. Mar. Biol. 112: 363–369

Millward, D. J., Garlick, P. J., Nnanyelugo, D. O., Waterlow, J. C. (1976). The relative importance of protein synthesis and breakdown in the regulation of muscle mass. Biochem. J. 156: 185–188

O'Connell, C. P. (1976). Histological criteria for diagnosing the starving condition in early post yolk-sac larvae of the northern anchovy, *Engraulis mordax*. Girard J. exp. mar. Biol. Ecol. 25: 285–312

O'Connell, C. P. (1981). Development of organ systems in the northern anchovy *Engraulis mordax* and other teleosts. Am. Zool. 21: 429–446

Ota, A. Y., Landry, M. R. (1984). Nucleic acids as growth rate indicators for early developmental stages of *Calanus pacificus Brodsky*. J. exp. mar. Biol. Ecol. 80: 147–160

Pedersen, B. H., Nilssen, E. M., Hjelmeland, K. (1987). Variations in the content of trypsin and trypsinogen in larval herring (*Clupea harengus*) digesting copepod nauplii. Mar. Biol. 94: 171–181

Pedersen, B. H., Ugelstad, I., Hjelmeland, K. (1990). Effects of a transitory low food supply in the early life of larval herring (*Clupea harengus*) on mortality, growth and digestive capacity. Mar. Biol. 107: 61–66

Raae, A. J., Opstad, I., Kvenseth, P., Walther, B. T. (1988). RNA, DNA and protein during early development in feeding and starved cod (*Gadus morhua* L.) larvae. Aquaculture, Amsterdam 73: 247–259

Richard, P., Bergeron, J.-P., Boulhic, M., Galois, R., Person-Le Ruyet, J. (1991). Effect of starvation on RNA/DNA and protein content of laboratory reared larvae and juveniles of *Solea solea*. Mar. Ecol. Prog. Ser. 72: 69–77

Robinson, S. M. C., Ware, D. M. (1988). Ontogenetic development of growth rates of larval Pacific herring, *Clupea harengus pallasi*, measured with RNA-DNA ratios in the Strait of Georgia, British Columbia. Can. J. Fish. aquat. Sciences 45: 1422–1429

Ueberschär, B. (1985). Untersuchungen über die Verdauungsenzyme von Fischlarven. Dipl.-Arbeit, Universität Kiel, Kiel

Ueberschär, B., Clemmesen, C. (1992). A comparison of the nutritional condition of herring larvae as determined by two biochemical methods – tryptic enzyme activity and RNA/DNA ratio measurements. ICES J. mar. Sci. 49: 245–249

Westerman, M. E., Holt, G. J. (1988). The RNA-DNA ratio: measurement of nucleic acids in larval *Scianops ocellatus*. Contr. mar. Sci. Univ. Texas (Suppl.) 30: 117–124

Wright, D. A., Martin, F. D. (1985). The effect of starvation on RNA/DNA ratios and growth in larval striped bass, *Morone saxatilis*. J. Fish. Biol. 27: 479–485

Communicated by O. Kinne, Oldendorf/Luhe